Evaluation of malaria screening during pregnancy with rapid diagnostic tests performed by community health workers in Burkina Faso, West Africa

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Keywords: *Plasmodium falciparum*, pregnancy, rapid diagnostic tests, community health worker

Abstract word count: 236

Manuscript word count: 3418

Number of tables: 8

Number of figures: 3

Number of supplemental files: 4

Abstract

One of the current strategies to prevent malaria in pregnancy is intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP). However, in order for pregnant women to receive an adequate number of SP doses, they should attend a health facility on a regular basis. In addition, SP resistance may decrease IPTp-SP efficacy. New or additional interventions for preventing malaria during pregnancy are therefore warranted. Because it is known that community health workers (CHW) can diagnose and treat malaria in children, in this study screening and treatment of malaria in pregnancy by CHWs was evaluated as an addition to the regular IPTp-SP program. CHWs used rapid diagnostic tests (RDT) for screening and artemether-lumefantrine was given in case of a positive RDT. Overall, CHWs were able to conduct RDTs with a sensitivity of 81.5% (95%CI 67.9 – 90.2) and high specificity of 92.1% (95%CI 89.9 – 93.9) compared with microscopy. After a positive RDT, 79.1% of women received artemether-lumefantrine. When treatment was not given, this was largely due to the woman being already under treatment. Almost all treated women finished the full course of artemether-lumefantrine (96.4%). In conclusion, CHWs are capable of performing RDTs with high specificity and acceptable sensitivity, the latter being dependent on the limit of detection of RDTs. Furthermore, CHWs showed excellent adherence to test results and treatment guidelines, suggesting they can be deployed for screen and treat approaches of malaria in pregnancy.

Background

Malaria in pregnancy can cause several adverse outcomes such as maternal anemia, stillbirths, miscarriage and low birth weight (reviewed by Desai *et al.*).[1] The current key strategy for prevention of malaria in pregnancy is intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP). IPTp-SP consists in the administration of SP during the second and third trimester when the women attend the antenatal care clinic (ANC),[2] and has proven effective in preventing placental malaria and low birth weight.[3] The WHO recommends that SP is administered at each antenatal care visit in the second and third trimester, provided it is at least a month apart from the previous dose.[2] However, IPTp-SP coverage of at least two doses is low in many Sub-Sahara African countries (on average 21.5%).[4] Reasons for low coverage are a lack of coordination and leadership, financial constraints, unmotivated and unsupported health staff, perceived risk of the medication, logistic challenges and ANC attendance among others.[5–7] Adolescents and primigravidae, who have among pregnant women the highest risk of malaria, are even less likely to receive sufficient doses of IPTp-SP, mainly due to low ANC attendance.[6] Besides the unsatisfactory uptake of IPTp-SP, SP resistance is widespread and rising in most sub-Saharan African countries.[8–10] In East Africa, where resistance is the highest of sub-Saharan Africa,[11] the efficacy of IPTp-SP seems already compromised.[12–15] Therefore, it is important to explore alternative or additional preventive measures.

Community case management of malaria (CCMm) aims at reducing malaria morbidity in children, by improving the access to diagnosis and treatment of malaria. CCMm relies on members of community, often named community health workers (CHW), who have been trained in diagnosing malaria with rapid diagnostic tests (RDTs) and administering an antimalarial treatment to positive tested children.[16] The strategy is effective as CHWs are capable of performing RDTs with a fair sensitivity (generally over 85% compared with microscopy).[17] Because of these positive results, an

intervention based on this strategy was considered for pregnant women. In this intervention CHWs were mobilized to screen pregnant women for malaria with RDTs at monthly intervals, between antenatal care visits.[18] Besides increasing the chance of detecting and treating malaria infections, this strategy also increases the total number of health-care contacts during pregnancy, something that is strongly encouraged by the WHO.[19] The performance of RDTs used by CHWs on pregnant women in rural Burkina Faso is reported here, including the adherence of CHWs and pregnant women to the test results and to treatment guidelines.

Methods

This study was nested in a cluster-randomized controlled trial (COSMIC; Trial Registration: Current Controlled Trials ISRCTN37259296 and clinicaltrials.gov NCT01941564) carried out in Benin, The Gambia and Burkina Faso, as described previously.[18] The aim of the main trial was to assess the efficacy of community screening and treatment of malaria during pregnancy on placental malaria. Here, the quality of RDT use by CHWs in pregnant women and adherence to treatment guidelines is described for the Burkina Faso study site.

Study procedures

The intervention of community screening and treatment of malaria during pregnancy was conducted in 15 villages in the Nanoro health centre catchment area, about 85 km North-West of Ouagadougou. Malaria is endemic in this region, but has a seasonal pattern with peak transmission occurring towards the end of the rainy season (that lasts from June until October). CHWs were instructed to pay monthly visits to pregnant women in their second and third trimester up to delivery. In each village there was a single CHW participating in the study, except for one village in which the initial CHW got a new job outside the study area and was therefore replaced. At each

home visit, CHWs performed a RDT (SD Bioline Ag-Pf), irrespective of symptoms, and if positive administered artemether-lumefantrine (COARTEM®). Treatment was not given if a woman reported to have received a course of artemether-lumefantrine in the past three weeks. Seriously ill women were referred to the health center. RDT results and any given treatment were recorded on a CRF by the CHW (supplemental file 1). In addition, the CHW collected a blood slide and blood spots on filter paper. All treated women were visited after three days by the CHW, to assess treatment adherence by administering a questionnaire and by checking the empty blisters. The number of tablets remaining and reasons for non-adherence were recorded in another CRF (supplemental file 2). Besides the home visits, pregnant women were encouraged by the CHWs to visit the ANC. At each ANC visit the woman received standard care, including IPTp-SP. Furthermore, a blood slide and filter paper were collected and clinical data and any given treatment was recorded on a CRF (supplemental files 3 and 4).

Study population and sample size

All women resident in the study area and without a known sensitivity to sulphonamides were eligible for inclusion in the COSMIC study. Pregnant women enrolled in intervention villages (*n* = 900) in Burkina Faso were included in the analyses of the current study.[18]

Community health workers

CHWs included in the study were already involved in community sensitization and organization of vaccination and malnutrition campaigns. Each of the CHWs was linked to a health facility. CHWs followed a program before the beginning of the trial in which they were trained in malaria symptoms and recognition of danger signs, the use of RDTs and the need and purpose of screening pregnant women for malaria. They were also explained the benefits of IPTp-SP and

advised to promote ANC visits and SP uptake among the pregnant women. CHWs were supervised by field workers.

Rapid diagnostic tests

RDTs used by the CHWs targeted the *P. falciparum* histidine-rich protein 2 (HRP2) antigen. A central stock of RDTs was kept at the Unité de Recherche Clinique de Nanoro (URCN) and the CHWs were supplied with small stocks at a regular basis. In case of an invalid test result, CHWs were instructed to repeat the RDT.

Microscopy

Filter papers and blood slides collected at home visits in intervention villages were transferred to the laboratory (URCN) on the same day. Blood slides were stained with Giemsa 3% for 45 – 60 minutes. Slides were read by two independent expert microscopists blinded to the RDT results. The number of parasites were counted against 200 leukocytes, or against 500 leukocytes if the count was <10 parasites/200 leukocytes. Slides were considered negative if no parasites were seen after examination of 100 high power fields. Any discrepancies between the two readings were resolved by consulting a third independent blinded reader.

Real-time PCR

Filter papers were air dried, sealed in bags with silica and stored at room temperature until shipment to the Netherlands (Academic Medical Centre, Amsterdam). For each selected filter paper a blood spot was punched out using acu-punch skin biopsy punchers (acuderm® inc, Florida, USA) and transferred to a 5 mL polystyrene tube with lysis buffer (bioMérieux, Marcy-l'Étoile, France). The

147 tubes were placed on a roller bank for 30 minutes. After lysis, the fluid was transferred to EasyMAG
148 vessels and Magnetic Bead Silica were added. DNA was extracted using the NucliSENS EasyMAG DBS
149 1.0 protocol (bioMérieux, Marcy-l'Étoile, France). Positive and negative controls were included
150 (blood spots from EDTA blood spiked with 3D7 or FCR3 culture and blood spots of uninfected EDTA
151 blood of the Dutch blood bank). Samples were stored at -20 ˚C.

152 Real-time PCR for detection of *Plasmodium falciparum* DNA was performed as previously
153 described with minor modifications.[20,21] In each reaction 2.5 µl of DNA, 5 mM MgCl2, 2.5 µl of 10x
154 PCR Buffer, 0.125 µl of HotStarTaq DNA Polymerase, 0.25 mM of each dNTP, 0.4 µM of each primer
155 and 0.1 µM of FAM-labelled probe ('5-aacaattggagggcaagg-3') was used. PCR Mix reagents were
156 ordered from Qiagen (Hilden, Germany) and all primers from Biolegio (Nijmegen, the Netherlands).
157 In each plate a dilution series of *P. falciparum* FCR3 culture was included (10^4 parasites/µL – 1
158 parasites/µL) as well as positive and negative DNA extraction controls and Milli-Q water. Reactions
159 were run on BioRad CFX real-time PCR machine with the following settings: initial denaturation 95°C
160 for 10 minutes, 40 cycles of 95°C for 60 seconds and 60°C for 20 seconds. Results were analyzed
161 using Bio-Rad CFX manager software (version 3.1).

162

163 *Statistical analyses*

164 All analyses were done using Stata 14.0. Microscopy was used as the reference test for RDT
165 performance. A sub-analysis at first home visit was done with real-time PCR as the reference test,
166 because it has been shown that sub-microscopic infections are also clinically important as they can
167 result in maternal anemia and preterm or low birth weight babies.[22] Sensitivity (proportion of
168 correctly identified positive samples), specificity (proportion of correctly identified negative
169 samples), positive predictive value (proportion of diseased after a positive RDT result; PPV), negative
170 predictive values (proportion of non-diseased after a negative RDT result; NPV) and prevalence
171 (proportion of positive test results of all tests performed) were calculated by using logistic regression

with robust standard errors to take clustering of tests within CHWs into account. For sensitivity and specificity analysis stratified by CHW, logistic regression with robust standard errors was used to account for repeated measurements of participants. However, for CHWs with 100% RDT sensitivity exact binomial confidence intervals were calculated since no between woman variation was observed. For comparisons of parasite density, Mann-Whitney U tests were performed for skewed data distributions.

Ethics

Informed consent was obtained for each participating community prior to the start of the trial. During the study informed consent was obtained for each participating woman. The study protocol was reviewed and ethically approved by the Institutional Ethics Committee of Centre Muraz in Burkina Faso on 19 September 2013 (ref A20-2013/CE-CM).

Results

Pregnant women were enrolled over a time period of 18 months; including follow-up the study lasted approximately 2 years in Burkina Faso (March 2014 – January 2016). A total of 900 women were recruited in 15 villages allocated to the intervention arm; of 861 women at least one home visit was recorded (Figure 1). The mean age of the women was 26 years olds (SD 6.3), with 10.5% of the women aged 18 or below. 19.2% (165/861) of women were primigravidae and 14.9% (128/861) secundigravidae. The modal number of visits per pregnancy was 3 (40.0%) though some women had up to 6 visits (Table 1). In total, 2516 home visits were done. There were 2,507 recorded RDT results, with 307 (12.2%) positive tests; 242 women tested positive at least once (up to a maximum of 4 times) (Figure 1). Of all microscopy slides, 147 of 2443 (6.0%) were positive. Over subsequent home visits, the proportion of positive RDTs was consistently higher than that of positive

196 microscopy (*p* values 0.011; <0.001; <0.001; 0.003; 0.017 for home visits 1 to 5 respectively) (Figure
197 2).

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199 *RDT versus microscopy*

200 Using microscopy as the reference test, RDT sensitivity was 81.5% (95%CI 67.9 – 90.2) and
201 specificity 92.1% (95%CI 89.9 – 93.9); positive predictive value (PPV) was 39.8% (95%CI 33.0 – 47.0)
202 and negative predictive value (NPV) was 98.7% (95%CI 97.6 – 99.3) (Table 2). When stratified by
203 home visit (Table 3), sensitivity ranged between 74.1 and 87.0% without a clear trend over
204 successive home visits ($p = 0.115$). However, specificity decreased over successive home visits (point
205 estimates decreased from 96.4 to 87.4%, $p < 0.001$). Consequently, the PPV differed significantly
206 between home visits ($p < 0.001$); a decrease in PPV was seen over successive home visits, except at
207 home visit 4 due to higher malaria prevalence by microscopy. The NPV remained high over
208 successive home visits without significant differences ($p = 0.086$)

209 Individual differences between CHWs in RDT sensitivity and specificity are presented in
210 Figures 3a and b. Sensitivity ranged from 27.3 up to 100% and specificity from 84.6 to 98.3%.
211 Variance between CHWs was significant for both sensitivity and specificity with intracluster
212 coefficients of 0.37 ($p < 0.001$) and 0.05 ($p < 0.001$) respectively. In particular, two CHWs (numbers
213 12 and 14) showed poor sensitivity (50.0%, 95%CI 15.8 – 84.2 and 27.3%, 95%CI 8.1 – 61.4
214 respectively). Due to the low overall prevalence the NPV remained high, 95.6% (95%CI 89.1 – 98.3)
215 and 93.2% (95%CI 86.3 – 96.7) for CHW 12 and 14 respectively. If the two CHWs were excluded from
216 the analyses, the overall sensitivity increased to 88.2% (95%CI 79.8 – 93.4) while the specificity
217 remained similar.

218 Discrepancies between RDT and microscopy results were further explored (Tables 4 and 5).
219 Parasite densities in microscopy positive slides (reference test) were compared between positive

(true positives) and negative RDTs (false negatives); for the former, the median parasite density was 2019.3 parasites/μL (IQR 703.5 – 4994.0) while it was 104 parasites/μL (IQR 72.0 – 530.5) for the latter ($p < 0.001$) (Table 4). Almost half of the women (48.9%, 88/180) with RDT positive and microscopy negative results (false positives) had taken anti-malarial treatment (AL, SP or quinine) within the two weeks before the RDT testing was performed; this was 62.6% (113/180) when considering the previous four weeks. When including reported but unproven treatment, the proportions increased to 56.1% (101/180) and 72.8% (131/180) respectively (Table 5).

RDT versus real-time PCR

From first home visit, 628 RDTs were available for analyses. Malaria prevalence by real-time PCR was 6.0% (95% CI 4.3-8.1%). When taking real-time PCR as reference test and after correction for clustering, RDT sensitivity was 75.7% (95% CI 66.2 – 83.2) and specificity 96.6% (95%CI 94.2 – 98.0) (Table 3).

Median parasite density (by real-time PCR) was 34.65 parasites/μL (IQR 10.4 – 111.3) in true positive RDTs and 1.12 parasites/μL (IQR 0.5 – 20.6) in false negative RDTs ($p = 0.04$) (Table 6). Of women with a false positive RDT, 40% (8/20) had used anti-malarial treatment within the last four weeks, when including reported but unproven treatment this increased to 55% (11/20) (Table 7).

Adherence to test results by CHWs and pregnant women

Of RDT positive women 79.1% (239/302) were treated with artemether-lumefantrine by the CHW. The most common reason for not giving treatment despite a positive RDT was ongoing treatment (77.9%). Furthermore, in 4 cases the CHW reported that he/she had no artemether-lumefantrine in stock. For the remaining 11 cases, the reason for non-adherence to the treatment protocol is unknown (Table 8). Full adherence to the drug regimen by pregnant women was 96.5%

(no anti-malaria tablets left after three days). Reasons for non-adherence were side-effects (3/7), the woman forgot to take the medication (1/7) or the woman was already under treatment (1/7) (Table 8).

Discussion

CHW are able to screen pregnant women for malaria with RDTs and treat them adequately if positive. CHWs were able of performing RDTs with a fair sensitivity of 81.5% (95%CI 67.9 – 90.2) and specificity of 92.1% (95%CI 89.9 – 93.9). Previous studies on HRP2-based RDTs performed by professional health care showed higher sensitivity (94%, 95%CI 91 - 96) on average, but lower specificity (81%, 95%CI 71 – 88) in pregnant women (reviewed in Kattenberg *et al.*).[23] These differences can be related to RDT brands used, endemic settings, or skills in execution of RDTs. The latter could also be the cause of CHWs not doing equally well in terms of RDT sensitivity in this study, with 2 CHWs (12 and 14) performing unsatisfactorily (sensitivities of 50% and 27% respectively). However, the CHWs were supervised at a regular basis during the study and no failures in RDT execution were reported by the field supervisors. Therefore, it remains unclear if this was a problem of RDT execution, or if there are other reasons. Previous studies have shown that mistakes in RDT execution are often related to the volume of blood and buffer used, the timing of reading, and/or incorrect interpretation of faint bands or invalid results.[17]

False negative RDT results may be explained by the detection threshold of the test (around 200 parasites/µL),[24,25] as almost 60% of all false negatives had a lower density. The large majority (8/11) of RDT false negatives above the 200 parasites/ µL threshold, were missed by the two poorer performing CHWs, again suggesting mistakes in the execution of the tests. However, even if only well performing CHWs would screen pregnant women for malaria, it means that some women with low parasite densities would be left untreated. This is unfortunate since it has been shown that infections with low parasite densities are also related to maternal anemia, low birth weight and

premature births.[22] Furthermore, the sensitivity of RDTs was calculated against microscopy of peripheral blood, while both these methods (as well as real-time PCR of peripheral blood) may miss placental infections.[23] Therefore, the number of women with a malaria infection but not identified by a RDT is likely higher than presented here.

Most false positive RDT results can probably be attributed to prolonged antigen circulation after clearance of a *P. falciparum* infection. While microscopy detects live parasites that are usually cleared within a few days after treatment, it has been shown that HRP2 antigens can persist in the circulation for at least 4 weeks after treatment in pregnant women.[20] This explains the decreasing specificity over successive home visits; it reflects the increased chance of women having experienced a malaria infection from which HRP2 antigens are still circulating. In our study, 72.6% of the women had used or reported use of anti-malarial treatment in the 28 days preceding a false positive RDT. For the remaining false positive results, it could be that reporting anti-malarial treatment was not always accurate, resulting in a recall bias. It could also be that some false positive results were actually true positives, but missed by microscopy reading. This seems to be the case for some positive RDTs at home visit 1 that were tested negative by microscopy but positive by real-time PCR.

The comparisons of RDT with real-time PCR resulted in a lower sensitivity 75.7 % (95% CI 66.2 – 83.2) than with microscopy as reference test. Given that real-time PCR can detect even lower parasite densities than microscopy (~20 parasites/mL versus 50 – 100 parasites/µL respectively), this is to be expected.[21,25] Compared with two other studies in Burkina Faso in which pregnant women were tested at antenatal care visits by professional health care workers, the sensitivity was high compared with one (sensitivity 55.8 %, 95%CI 50.0 – 62.4),[26] but low compared with the other (90.9%, 95%CI 87.5, 93.6).[27] However, both used a different test for comparison (nested PCR and not real-time PCR) which could impact on sensitivity; in addition, the latter study used both PCR and microscopy as reference test. The observation that parasite densities were significantly lower for

false negative RDT samples than for true positive RDT samples in our study, confirmed the idea that the major bottleneck was the detection limit of RDTs.

The specificity of RDTs compared with real-time PCR was high (96.6%, 95%CI 94.2 – 98.0) and fairly similar to specificity found in the two previous studies in Burkina Faso (99.3%, 95%CI 98.4–99.7 and 94.1%, 95%CI 89.4, 97.1).[26,28] Antigen persistence of a cleared infection may again be the cause of the few false positive RDT results, because in contrast to antigen, DNA from dead *Plasmodium* parasites seems to be rapidly cleared from the bloodstream.[29]

Adherence to test results by CHWs was excellent, given the fact that almost 80% of the women were treated after a positive RDT, and that the most common reason for not giving treatment was that the woman was already under treatment or had just finished treatment. This shows that CHWs were well trained in treatment guidelines and unlikely to over-treat the pregnant women. Good adherence to positive test results was shown in previous studies on CHWs.[17] Furthermore, the high adherence of pregnant women to the full course of AL shows the high trust in the CHWs and the test results, at least within this trial context.

A limitation of this study was the quality of filter paper samples. While most CHWs seemed sufficiently trained in performing RDTs, the correct preparation of filter papers turned out to be more difficult. For most blood spots the amount of blood was less than the requested 50 µL for which the extraction and real-time PCR were validated. The lack of sufficient blood may have led to wrong estimations of parasite density and to false negative real-time PCR results if the parasite density was already low. However, because specificity was high for RDTs compared with real-time PCR as reference test, it is unlikely that the latter was an issue.

Furthermore, it is unclear whether the screen and treat intervention by CHWs would work as well if it was implemented in the regular health care system, as the current results were obtained during a trial setting in which stock supply was carefully managed and CHWs were in close contact with field supervisors. This is something that should be evaluated after implementation. This study

has highlighted the qualities and the issues of screening pregnant women with relatively simple diagnostics for malaria by CHWs. CHWs can perform RDTs with acceptable sensitivity and high specificity and have shown good adherence to treatment guidelines. The biggest area for improvement, before implementing this intervention, would be thorough examination of correct execution of RDTs by all CHWs. Due to the intrinsic limitations of the current RDTs, cases with low parasite densities will nevertheless be missed. A new simple diagnostic point-of-care test, that can detect lower parasite densities and that is preferably less sensitive to antigen persistence, could therefore further improve overall performance. In any case, this study has shown that CHWs can be trained and instructed for innovative purposes, which might present new opportunities for other public health issues.

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331 Table 1. Characteristics of pregnant women with at least one home visit

Participant characteristics (*n* = 861)		
Age, mean±SD, (median, IQR)	26±6.3	(26, 21 - 30)
Gravidity		
Primigravidae, % (nr)	19.2	(165)
Secundigravidae, % (nr)	14.9	(128)
Multigravidae, % (nr)	66.0	(568)
Nr. of home visits per woman, % (nr)		
1	9.3	(80)
2	22.8	(196)
3	40.0	(344)
4	22.9	(197)
5	4.6	(40)
6	0.5	(4)

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Table 2. RDT performance compared with microscopy; sensitivity, specificity, PPV and NPV

RDT versus microscopy (*n* = 2434)	Microscopy positive	Microscopy negative	Total
RDT positive	119	180	299
RDT negative	27	2108	2135
Total	146	2288	
Sensitivity % (95% CI)	81.5 (67.9 – 90.2)		
Specificity % (95% CI)	92.1 (89.9 – 93.9)		
Positive predictive value % (95% CI)	39.8 (33.0 – 47.0)		
Negative predictive value % (95% CI)	98.7 (97.6 – 99.3)		

Table 3. RDT sensitivity and specificity compared with microscopy and real-time PCR, stratified for home visits 1 - 4

	Home visit 1	Home visit 2	Home visit 3	Home visit 4	*p* value
RDT versus microscopy, *n*	837	759	567	228	
Sensitivity (95% CI)	78.6 (62.6 – 88.9)	87.0 (71.9 – 94.6)	74.1 (48.1 – 89.8)	82.8 (55.3 – 94.9)	0.115
Specificity (95% CI)	96.4 (94.4 – 97.7)	90.3 (86.9 – 92.9)	91.1 (87.8 – 93.6)	87.4 (80.6 – 92.1)	0.000
Positive predictive value % (95% CI)	53.2 (39.2 – 66.7)	36.7 (28.0 – 46.3)	29.4 (20.4 – 40.3)	49.0 (34.2 – 64.0)	0.000
Negative predictive value % (95% CI)	98.8 (97.7 – 99.4)	99.0 (97.9 – 99.6)	98.6 (96.5 – 99.5)	97.2 (91.3 – 99.1)	0.086
RDT versus qPCR, *n*	621	*NA*	*NA*	*NA*	
Sensitivity (95% CI)	75.7 (66.2 – 83.2)	*NA*	*NA*	*NA*	*NA*
Specificity (95% CI)	96.6 (94.2 – 98.0)	*NA*	*NA*	*NA*	*NA*
Positive predictive value % (95% CI)	58.3 (42.3 – 72.8)	*NA*	*NA*	*NA*	*NA*
Negative predictive value % (95% CI)	98.4 (97.5 – 99.0)	*NA*	*NA*	*NA*	*NA*

Home visit 5 and 6 not presented because of small sample sizes (*n* = 39 and *n* = 4 respectively)

Table 4. False negative RDT results (microscopy as reference test): real-time PCR results and parasite density

Discrepancy	*N*	PCR positive	Parasite density*
False negative RDT (ref microscopy)	27 (1.1%)	2/6 (33.3%)	16 had parasite density <200 p/µL (59.3%) Median: 104, IQR: 72 – 530.5 p/µL

*by microscopy

Table 5. False positive RDT results (microscopy as reference test): real-time PCR results and recent anti-malarial treatment

Discrepancy	*n*	PCR positive (HV1)	AL treatment	IPTp-SP	Any treatment
False positive RDT (ref microscopy)	180 (7.4%)	6/24 (25%)	50 had received AL in last 14 days (27.8%) 72 had received AL in last 28 days (40.0%) 47 reported being under treatment with AL	49 had received SP in last 14 days (27.2%) 64 had received SP in last 28 days (35.6%)	88 had received AL, SP or quinine in last 14 days (48.9%) 113 had received AL, SP or quinine in last 28 days (62.8%) 101 reported or received AL, SP or quinine in last 14 days (56.1%) 131 reported or received AL, SP or quinine in last 28 days (72.8%)

Table 6. False negative RDT results (real-time PCR as reference test): microscopy results and parasite density

Discrepancy	*n*	Microscopy positive	Parasitemia* by qPCR
False negative RDT (ref real-time PCR)	9 (1.4%)	2/8 (25%)	All had parasitemia <200 p/µL Median: 1.1, IQR: 0.47 – 20.6 p/µL

*by qPCR

Table 7. False positive RDT results (real-time PCR as reference test): microscopy results and recent anti-malarial treatment

Discrepancy	*n*	Microscopy positive	AL treatment	IPTp-SP	Any treatment
False positive RDT (ref real-time PCR)	20 (3.2%)	2/20 (10%)	4 had received AL in last 14 days (20%) 4 had received AL in last 28 days (20%) 6 reported being under treatment with AL	4 had received SP in last 14 days (20%) 4 had received SP in last 28 days (20%)	8 had received AL or SP in last 14 days (40%) 8 had received AL or SP in last 28 days (40%) 11 reported or received AL, SP or quinine in last 14 days (55%)

Table 8. Adherence to treatment guidelines after a positive RDT by CHWs and pregnant women

RDT positive	AL given	Reasons non-adherence CHW	AL course completed	Reasons non-adherence full AL course
n = 307	239/302 (79.1%)	4/46 no AL available 53/68 already under treatment (77.9%)	195/202 (96.5%)	3/7 medicine made woman feel ill 1/7 forgot to take medicine 1/7 was already under treatment with AL 2/7 unknown

Acknowledgements

We would like to thank all study participants of the COSMIC trial and the research and field staff of the Unité de Recherche Clinique de Nanoro. Also we would like to thank K. Peeters Grietens and A. Compaore for critically reviewing the manuscript. Furthermore, we would like to thank MR4 for providing us with malaria parasites contributed by ATCC®.

Financial support

This study was funded by European Community's Seventh Framework Programme under grant agreement No. 305662 (Project: Community-based scheduled screening and treatment of malaria in pregnancy for improved maternal and infant health: a cluster-randomized trial 'COSMIC').

Disclosures

The authors declare that they have no competing interests.

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